STUDIES OF COMMERCIAL SAUERKRAUT WITH SPECIAL REFERENCE TO CHANGES IN THE BACTERIAL FLORA DURING FERMENTATION AT LOW TEMPERATURES[1]

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INTRODUCTION

The fermentation of cabbage is a spontaneous one, and the manufacturer of sauerkraut must take the undesirable microorganisms along with the desirable ones. Because of this chance inoculation, there is a wide variation in the quality of sauerkraut. A large variety of organisms is introduced with the green plant tissue when this is placed in the vats. Many of the forms on plants can not survive under the conditions in the vat, while others find the conditions very favorable and increase to enormous numbers.

Changes in oxygen tension, hydrogen-ion concentration, and fermentable carbohydrate bring about conditions which for a few days are favorable for certain kinds of bacteria, but within a few days more these conditions may change and become unsuitable for that particular kind of microorganism. A new flora better adapted to the new conditions may come to the front and persist for a shorter or longer period of time. Although the general character of the fermentation is similar in all vats, no two are exactly the same.

The extensive research which has been made on silage shows clearly that the souring of plant tissue is the result of the combined action of many types of microorganisms. Although there are decided differences between the formation of silage and of sauerkraut, the main outlines of the two processes are the same and many of the same bacteria are found in both fermentations.

REVIEW OF LITERATURE

In the older literature on sauerkraut undue emphasis probably was placed on the activities of one type of microorganism. Conrad (*4*),[2] the first investigator in this field, isolated a culture which he regarded as the principal agent in the fermentation and to which he gave the name *Bacterium brassicae acidae*. It was a motile, Gram-negative rod which, among other products, produced hydrogen and methane. Most of the bacteria which have since been obtained from sauerkraut are nonmotile, Gram-positive, and produce neither hydrogen nor methane. *Bacterium brassicae acidae* is clearly not the usual kind of bacteria which occurs in sauerkraut.

In 1903 and 1905 Wehmer (*23*, *24*) published two papers on several aspects of sauerkraut fermentation. He concluded that gas formation was due to alcohol-forming yeasts and that acid production was caused by an organism which he tentatively named *Bacterium brassicae*. This organism was a short rod which at times appeared to be almost

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[2] Reference is made by number (italic) to "Literature cited," p. 94.

a coccus and did not form any gas. Wehmer gives such a meager description of his organism that it is practically impossible to identify it with any culture which has since been isolated from sauerkraut. In a recent publication LeFevre (*12*) has concluded that *Bact. cucumeris fermentati*, isolated by Henneberg (*9*), and *Lactobacillus pentoaceticus*, isolated by Fred, Peterson, and Davenport (*5*), are identical with Wehmer's *Bact. brassicae*. While Wehmer says nothing of the products formed by *Bact. brassicae* other than that it formed acid, he repeatedly emphasizes the fact that *it did not form gas*. Gas production is an outstanding characteristic of *L. pentoaceticus*. Approximately 25 per cent of the glucose and other sugars is converted into carbon dioxide. Because of this and other differences it seems certain that *Bact. brassicae* and *L. pentoaceticus* are different microorganisms.

About the same time that the papers of Henneberg and Wehmer appeared, Butjagin (*3*) published a paper in which he described the successive changes in the flora of sauerkraut. He isolated and gave a good description of a short round-ended rod which in many respects resembled *Bacterium guntheri* of Gunther and Thierfelder. Aderhold (*1*) also isolated a similar organism from pickle and sauerkraut fermentations. Butjagin's bacterium differed from *Bact. guntheri* in that it did not grow well in milk and coagulated milk only after 21 to 23 days. It was Gram-positive, nonmotile, and produced no gas. In respect to the last two characteristics it was like Wehmer's *Bact. brassicae*. Wehmer makes no mention of the reaction of his organism to Gram's stain, but it is probable that it was positive. Probably both organisms are members of a large group of bacteria occurring in silage, sauerkraut, and pickles, which are strong acid producers but do not form gas. A detailed examination of the reaction of the non-gas-producing sauerkraut bacteria toward various sugars and other organic compounds shows marked differences among the various members with respect to their ability to ferment these compounds.

Shortly before Wehmer's long paper on sauerkraut appeared, Perekalin (*14*) published a short note about an acid-tolerant bacterium which he had isolated from sauerkraut. This organism was a short, nonmotile rod, negative to Gram's stain, and did not produce spores nor coagulate milk.

In 1909 Gruber (*8*) isolated an acid-producing culture which he believed to be characteristic of sauerkraut. This organism resembled Conrad's *Bacterium brassicae acidae*. It was motile, produced gas, and in other respects resembled the colon type of bacteria. He named his organism *Pseudomonas brassicae acidae*.

Round (*17*) published a short note in 1916 regarding the large numbers of bacteria in commercial suaerkraut, and concluded that bacteria alone are responsible for the fermentation. An organism which he isolated was studied by Fred, Peterson, and Davenport (*5*) in connection with the fermentation of xylose. These investigators isolated a large number of similar organisms from silage, sauerkraut, soil, manure, and cereals. They belong to the so-called "mannitol-forming" bacteria, and have received considerable attention in connection with the production of wine and butyl alcohol. In wine they produce a sour, bitter taste and in the butyl-alcohol fermentation they inhibit the growth of the organism which produces butyl alcohol.

In 1916 Henneberg (*10*) published a series of papers in which he discussed the manufacture of sauerkraut from many different angles. He divided the bacterial flora of sauerkraut into 9 or 10 groups. In the early days of the fermentation, bacteria of the colon-aerogenes type may be present, but Henneberg does not believe that they serve any useful purpose and should be excluded if possible. Oval-shaped forms, similar to *Streptococcus lactis*, are also frequently found in the early stages of the fermentation, but these gradually die off as a considerable degree of acid is attained. Henneberg attributes the odor and taste of sauerkraut chiefly to the activities of alcohol, acetic-acid-producing lactics such as *Bacterium brassicae fermentatae*. Other aroma-producing bacteria are the Pediococcus forms, of which there are many varieties. Particularly abundant in sauerkraut are the short rod forms to which group the incompletely described *Bact. brassicae* of Wehmer belongs. In old, strongly acid sauerkraut *Bact. cucumeris fermentati* and a gas-producing variety of this form are common. Other bacteria, which are less clearly defined, are chain types, flocculating types, and slime-producing types.

The functions of yeasts in the production of sauerkraut is still an unsettled question. Wehmer attached great importance to their presence, while Butjagin suggested that they might be more or less harmless, nonessential organisms. The esterlike flavors have been attributed to the compounds formed between the alcohols produced by the yeast and the acids produced by the bacteria. Peterson, Fred, and their associates (*16*, *21*) have shown that the mannitol-forming bacteria convert 25 per cent of the glucose and other sugars into ethyl alcohol. It is therefore not necessary to have yeasts in sauerkraut in order to have alcohol present.

Fred and Peterson (*6*) also showed that the so-called "pink" sauerkraut is due to yeasts. Under certain conditions the sauerkraut may become infected with large numbers of wild yeasts which produce a dirty brown color and an offensive odor and taste. On the basis of existing data no final conclusion can be drawn as to the rôle of yeasts in sauerkraut fermentation.

In 1925 Brunkow, Peterson, and Fred (*2*) published the results of a systematic study of the number and kinds of bacteria which occur in experimental sauerkraut at successive times during the fermentation period. It was desired to extend this study to commercial sauerkraut and to make a somewhat more thorough investigation of the different forms, especially as regards the occurrence of the mannitol-forming bacteria. The present paper deals with the results of this investigation.

METHODS

SOURCE OF SAMPLES

Most of the samples of sauerkraut juice were secured from a commercial sauerkraut factory, which will be designated as factory A. The samples were taken from four large vats, each vat containing about 40 tons of sauerkraut. Spigots were placed on the side near the bottom of the vats. This made it possible to draw off the juice as needed without disturbing the sauerkraut. Before a sample was taken to be analyzed some of the juice was allowed to flow through the spigot. At the beginning of the fermentation samples

were drawn at frequent intervals, but in the last part of the fermentation period samples were taken about every 30 days.

Samples of sauerkraut juice which had been in the vats from 41 days to 1 year were obtained from another factory, B.

TEMPERATURE OF VATS, ACIDITY OF SAUERKRAUT, AND NUMBER OF BACTERIA

To obtain the temperatures at which fermentation took place, a pointed metal tube was forced into the sauerkraut in such a way that the lower end was approximately at the center of the vat. Inside the tube was a thermometer, the lower part of which was inserted through a cork into a slender test tube filled with water. By this arrangement no apparent change in temperature took place for several minutes after the thermometer was withdrawn from the tube. The temperature was also taken of the sample as it was drawn from the spigot. The two temperature readings varied only a few degrees.

A 10 c. c. sample of the sauerkraut juice was diluted with an equal volume of water and, after boiling for a few seconds to expel the CO_2, was titrated with 0.1 N NaOH to the end point of phenolphthalein.

Counts of the number of bacteria were made by plating the sauerkraut juice on glucose-yeast-water agar which had been adjusted to PH 6.8. The plates were incubated at 28° C. and counted after 48 hours and again after 96 hours of incubation. After counting, 10 colonies were picked from one of the plates from each vat. As a rule the picking was made from the plate on which from 30 to 50 colonies were growing. The plate was sectioned off until there were about 10 colonies to the section. The section which appeared to have the most representative growth was selected and *all the colonies picked.* In this way 80 cultures were obtained from each of the four vats. Forty cultures were picked from the four samples of 41-day sauerkraut obtained from factory B and 10 cultures from the 1-year-old sauerkraut; in all, 370 cultures were picked.

DISCUSSION OF DATA

The temperature, acidity, and number of bacteria in the vats at various times during the fermentation are shown by the curves in Figures 1 to 4. The outstanding fact regarding these sauerkrauts is the low temperature at which fermentation took place. At the time the vats were filled the temperature was almost freezing, and at no time during the fermentation did it rise above 10.5° C. Most sauerkraut manufacturers would regard these as unusually low temperatures, but it must be noted that Wehmer considers 6° to 8° C. as the proper temperature at which to make sauerkraut. That the low temperature reduced the number of bacteria is evident from the curves in Figures 1 to 4. The highest number of bacteria in a normal fermentation is usually found from about the fifth to the seventh day. In the four vats studied the maximum was not reached until between the fifth and the twentieth day. Vat 3, which had the lowest average temperature, had an unusually low count throughout the entire fermentation, and the maximum number was not obtained until the twentieth day.

After the maximum was reached the count for all the vats rapidly dropped to a small number and continued at this low level for several weeks. After 90 days the temperature of the vats rose slightly, and

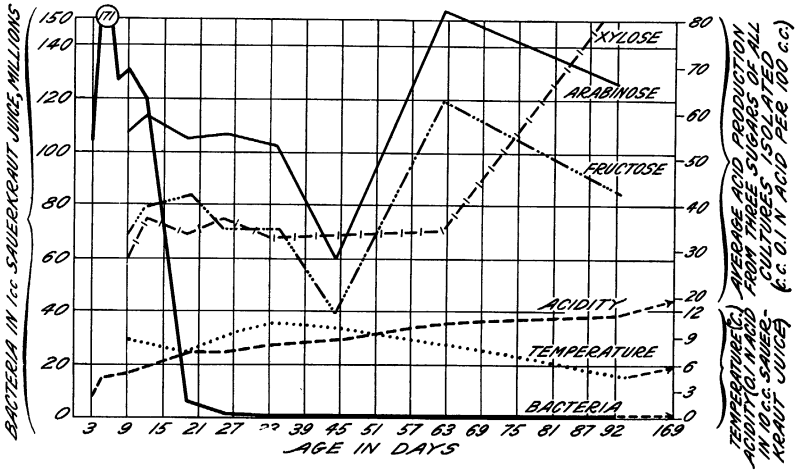

FIG. 1.—Temperature, acidity, and number of bacteria at various times during the formation of sauerkraut; also the acidity produced from certain sugars by sauerkraut bacteria. (Vat 1)

this rise was accompanied by a small increase in the number of bacteria.

In the first days of the fermentation numerous peptolytic catalase-positive bacteria were present, but these soon disappeared. Coccus

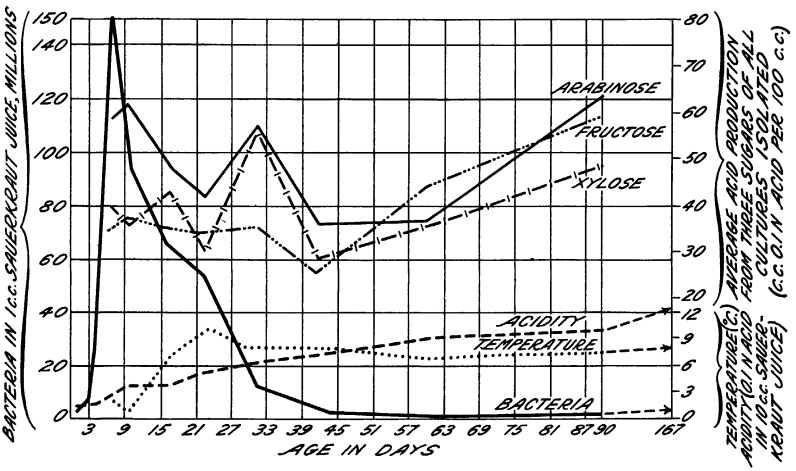

FIG. 2.—Temperature, acidity, and number of bacteria at various times during the formation of sauerkraut; also the acidity produced from certain sugars by sauerkraut bacteria. (Vat 2)

forms predominated in the early stages of the fermentation; short rods were present all the time, but were more abundant during the latter part, and long rods appeared only toward the end of the fermentation. All four vats showed a slow increase in acid, but none

reached a particularly high amount. At the end of 90 days about 1 per cent of acid, calculated as lactic, was found. Samples of the sauerkraut juice were then analyzed for reducing sugars, and the

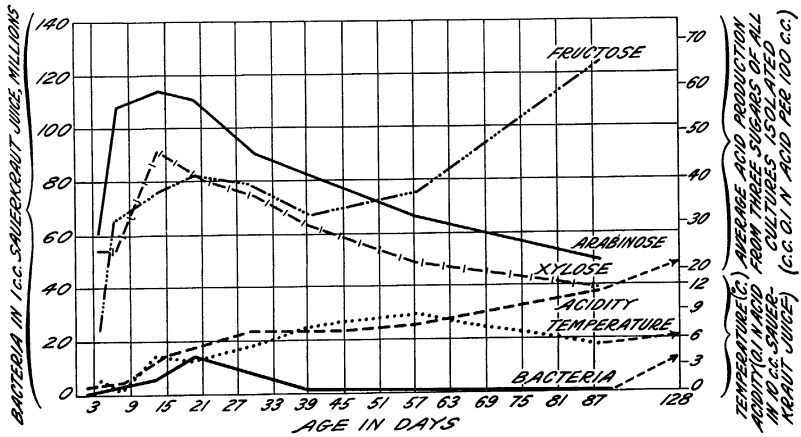

FIG. 3.—Temperature, acidity, and number of bacteria at various times during the formation of sauerkraut; also the acidity produced from certain sugars by sauerkraut bacteria. (Vat 3)

surprising fact was revealed that there was practically none left. Apparently complete fermentation had taken place at the low temperatures of the vats, but only a small part of the sugar had been converted into acids. A complete analysis of the sauerkraut juice

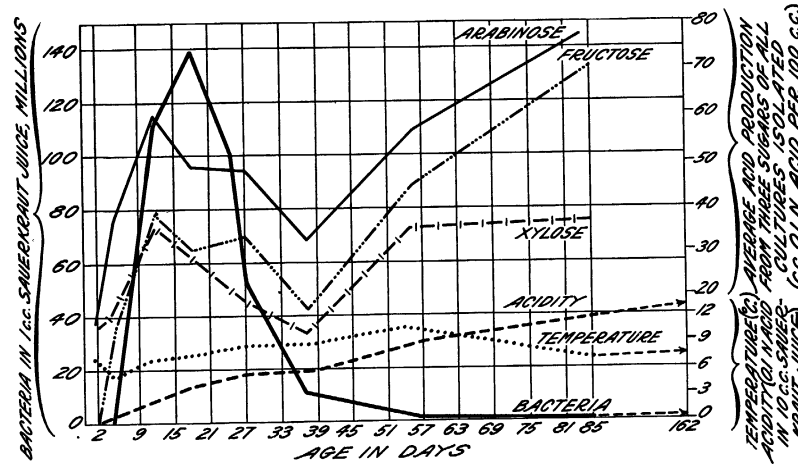

FIG. 4.—Temperature, acidity, and number of bacteria at various times during the formation of sauerkraut; also the acidity produced from certain sugars by sauerkraut bacteria. (Vat 5)

was therefore made to determine, if possible, what other fermentation products had been formed. The methods used have been described in previous publications (*18*, *19*, *5*, *15*).

TABLE 1.—*Fermentation products contained in sauerkraut juice from different vats (factory A)*

No. of vat	Temperature [a]	Age of sauerkraut	Sugar	Titratable acid as lactic	Volatile acid as acetic	Nonvolatile acid as lactic	Alcohol as ethyl
	° C.	*Days*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Vat 1	8.0	92	0.12	1.026	0.270	0.663	0.432
Vat 2	6.9	90	.10	.873	.266	.575	.653
Vat 3	5.3	87	.12	1.017	.276	.703	.920
Vat 5	7.7	85	.10	.972	.334	.678	.680

[a] Average for entire fermentation period, from 128 to 169 days.

FERMENTATION PRODUCTS

The percentage of volatile acid, nonvolatile acid, alcohol, and unfermented sugar contained in the sauerkrauts are given in Table 1. The percentage of acetic acid is normal but the amount of lactic acid is low. In a normal fermentation there is from three to five times as much lactic acid as acetic acid, but in the sauerkrauts studied the ratio is only from 2 to 2.5. The alcohol content ranged from 0.43 to 0.92 per cent, with an average of 0.67 per cent. The average for nine commercial sauerkrauts was found by Fred and Peterson (7) to be 0.47 per cent. The analytical data indicate that the low temperatures of these vats favored the development of alcohol-producing bacteria. It is possible that, if mannitol could have been determined on these samples, an unusually high percentage would have been found. No satisfactory determination could be obtained because of the interference of salt. When the vats were opened the sauerkraut was graded and was rated fair. From this limited evidence it would seem that low temperatures do not give the best quality of sauerkraut. More data must be obtained, however, before a final conclusion on this point can be drawn.

KINDS OF BACTERIA IN THE SAUERKRAUTS

A rough classification of the several hundred bacteria picked from the plates was made by inoculating 10 c. c. of 1 per cent yeast-water solutions of fructose, arabinose, and xylose with these cultures and determining the acidity formed after seven days of incubation. The titrations for the 10 cultures picked at each time of sampling were averaged for each sugar and the results plotted in the form of curves as shown in Figures 1 to 4.

While there are many irregularities in the curves, the acid production from these sugars seems to follow the bacterial curve during the first half of the fermentation. When the number of bacteria decreased, the acid-producing ability of the bacteria also decreased. The decline in acid production was not so great, however, as in numbers of bacteria, and after six weeks a general increase in acid production took place. A high acid-producing type of bacteria came to the front. These were probably the long rods which are usually abundant in old sauerkraut.

The cultures were also inoculated into litmus milk, but less than 50 per cent produced any reduction or curd in this medium and only a few digested milk. Those which did produce proteolytic changes in litmus milk were cultures which had been obtained in the early

stages of the fermentation. These bacteria were undoubtedly plant forms which did not find the conditions of the vat suitable for their existence.

The cultures were also tested for catalase with hydrogen peroxide, but more than 80 per cent were found negative. The great majority of bacteriâ in sauerkraut are catalase-negative, produce little or no change in milk, but ferment arabinose, xylose, and fructose readily with the production of considerable quantities of acid. The large number of pentose fermenters corroborates the findings reported in a previous paper (*2*) in which it was stated that the xylose fermenters practically equalled the number that attacked glucose.

FERMENTATION CHARACTERISTICS OF THE SELECTED CULTURES

On the basis of the above five tests 90 of the 320 cultures were selected for further study. In making this selection an effort was made to include representatives of all the various types encountered. Tables 2 to 5 give the representative types found in the four commercial vats at factory A. Table 6 contains the data for the selected cultures obtained from factory B. Numbers 60–2, 60–6, and 60–11 were selected from the year-old sauerkraut and all others in this table came from the 41-day sauerkraut. A few of the organisms in Table 6 are outstanding because of the great acidity developed in the sugars. Organisms in Tables 2 to 5, selected from factory A, where a low temperature was maintained, do not show such a marked ability to produce acid. Most of the cultures slowly produce acid in litmus milk and about half of them bring about some other change such as curdling, reduction, or granule formation. The mannitol formers produce less acidity and softer curds than the nonmannitol bacteria. Reduction of litmus is also more prominent with the latter group.

TABLE 2.—*Fermentation characteristics of the selected cultures from vat 1 (factory A)*

Culture No.	Litmus milk after 30 days	Gas from glucose	0.1 N acid in 100 c. c. of culture			
			Arabinose	Xylose	Glucose	Fructose
			C. c.	*C. c.*	*C. c.*	*C. c.*
40–1	No change	[a]+	72	54	78.9	50.0
40–3	do	+	62	22	75.8	47.2
40–6	do	+	76	50	73.1	48.4
41–2	do	+	72	50	75.9	71.6
41–10	do	+	78	48	75.9	46.8
43–8	do	+	78	50	84.8	52.0
44–1	do	+	88	62	75.9	53.6
45–3	do	+	69	50	86.0	50.0
46–2	do	+	51	115	96.0	58.0
46–8	Reduced	+	98	114	90.0	51.2
46–10	do	+	102	109	100.0	55.2
47–9	No change	+	106	120	94.0	50.4
48–1	do	+	36	112	80.0	49.0
48–3	do	+	134	107	82.0	50.0
48–6	Reduced	−	79	28	96.0	97.0

[a] Plus sign indicates presence of gas; minus sign, absence of gas.

TABLE 3.—*Fermentation characteristics of the selected cultures from vat 2 (factory A)*

Culture No.	Litmus milk after 30 days	Gas from glucose	0.1 N acid in 100 c. c. of culture			
			Arabinose	Xylose	Glucose	Fructose
			C. c.	*C. c.*	*C. c.*	*C. c.*
50–2	No change	+	82	52	81.8	52.0
50–5	do	+	72	66	85.0	50.4
51–9	Soft curd	+	86	50	82.0	53.2
53–4	No change	+	24	68	81.8	52.8
53–5	do	+	70	58	58.4	51.6
53–8	do	+	22	78	80.5	52.0
54–1	do	−	18	26	54.0	54.0
54–5	do	+	74	56	82.4	56.4
54–10	Granules	−	74	24	62.4	73.0
55–4	No change	+	90	52	84.0	52.0
55–6	do	+	32	76	82.0	51.6
55–9	do	+	80	80	88.0	54.4
56–2	Reduced	+	76	59	102.0	52.4
56–7	No change	−	15	16	86.0	32.8
57–2	Reduced	−	19	21	98.0	69.2
57–3	do	−	120	28	90.0	60.8
57–7	do	+	115	114	86.0	45.2
57–8	No change	+	67	60	88.0	47.2
58–3	do	−	22	18	96.0	82.0
58–6	do	+	120	128	102.0	78.0
58–8	Reduced	−	106	32	104.0	104.0

TABLE 4.—*Fermentation characteristics of the selected cultures from vat 3 (factory A)*

Culture No.	Litmus milk after 30 days	Gas from glucose	0.1 N acid in 100 c. c. of culture			
			Arabinose	Xylose	Glucose	Fructose
			C. c.	*C. c.*	*C. c.*	*C. c.*
21–1	No change	+	64	54	76.0	50.4
21–2	Reduced, curd	+	36	22	54.0	4.8
22–3	No change	+	82	26	77.4	48.4
22–5	do	+	94	54	90.5	47.6
22–8	do	+	72	46	62.8	93.2
22–10	Peptonized	+	34	40	53.1	8.0
24–1	No change	+	80	74	74.4	54.4
25–6	do	+	86	60	80.5	55.6
25–7	Reduced, curd	−	76	70	65.6	71.6
26–4	Reduced	+	62	46	84.0	62.4
26–8	No change	+	64	59	57.2	90.0
27–1	Reduced	+	63	44	78.0	49.2
27–8	do	+	78	62	84.0	45.2
28–5	No change	−	59	19	64.0	60.4
28–7	Reduced	+	66	104	80.0	58.4
28–8	No change	−	19	25	88.0	67.6
29–1	Reduced	−	66	38	94.0	99.0
29–4	No change	+	127	115	104.0	73.0
29–6	do	−	21	19	104.0	97.0
29–7	do	−	28	22	82.0	99.0

TABLE 5.—*Fermentation characteristics of the selected cultures from vat 5 (factory A)*

Culture No.	Litmus milk after 30 days	Gas from glucose	0.1 N acid in 100 c. c. of culture			
			Arabinose	Xylose	Glucose	Fructose
			C. c.	*C. c.*	*C. c.*	*C. c.*
30–2	Reduced, curd	+	42	30	39.4	3.6
30–7	Reduced	+	27	48	50.6	11.6
31–1	No change	+	94	24	74.5	48.0
31–2	Reduced, curd	−	10	28	55.0	12.0
31–8	do	+	40	38	40.8	8.4
32–2	do	−	70	70	73.6	57.6
32–6	No change	+	88	50	73.1	55.2
33–1	do	+	72	50	50.0	70.1
33–2	do	+	72	44	71.6	50.8
34–2	Reduced	+	80	42	90.0	51.2
34–4	do	+	53	39	88.0	51.2
34–7	do	+	66	76	84.0	47.6
35–1	No change	−	65	17	74.0	22.8
35–7	Reduced	+	78	52	90.0	48.8
35–10	do	+	61	42	86.0	50.8
36–2	Reduced, curd	−	81	35	100.0	95.6
36–4	Reduced	+	108	133	100.0	51.6
36–6	Reduced, curd	−	23	34	108.0	88.0
37–2	Reduced	−	109	28	104.0	94.0
37–3	do	+	70	48	92.0	91.0
37–6		+	102	125	88.0	75.0

TABLE 6.—*Fermentation characteristics of the selected cultures from factory B*

Culture No.	Litmus milk after 30 days	Gas from glucose	0.1 N acid in 100 c. c. of culture			
			Arabinose	Xylose	Glucose	Fructose
			C. c.	*C. c.*	*C. c.*	*C. c.*
60–2	Reduced	+	120	116	86	106
60–6	do	+	84	105	98	54
60–11	Reduced, curd	−	27	25	106	100
62–3	do	−	20	23	90	96
62–6	No change	+	120	109	126	76
62–10	Reduced, curd	−	73	39	78	101
63–5	Reduced	+	119	109	96	71
64–2	Reduced, curd	−	69	30	100	91
64–7	Reduced	+	118	22	84	118
64–8	do	−	20	24	88	154
64–9	Reduced, curd	+	114	106	92	87
65–5	do	−	66	34	98	201
65–9	Reduced	−	19	22	90	152

GAS FORMATION

The cultures were inoculated into 10 c. c. of 1 per cent glucose-yeast water, sealed with sterile melted vaseline and allowed to ferment at 28° C. They were observed from day to day for gas formation. If the vaseline plug was pushed up they were recorded as gas-positive. If the vaseline remained at the surface of the liquid and there was no apparent leakage of gas, they were called negative. At the end of seven days the vaseline plug was removed and the culture titrated for acidity with 0.1 N. NaOH. If acid had developed it was evident that the organisms had grown and the negative results were not due to a failure to grow.

Of the 90 cultures, 63 formed gas and 27 did not. The proportion of gas-forming organisms was higher in the vats from factory A than

from factory B. In the first factory there were almost three times as many gas formers as nongas formers, while in the second factory the numbers were about equal. Low temperatures appear to favor the development of gas-producing bacteria. As will be shown later, gas and mannitol formation coincide in most cases. Certain cultures, however, produced no gas but formed a small quantity of mannitol.

Reaction to Gram's Stain

In most cases the gas-producing organisms were also positive to Gram's stain. Most of the organisms were short rods, but some of those picked from the young sauerkraut were cocci. In the later stages of the fermentation a considerable number of long rods appeared.

RESELECTED AND PURIFIED CULTURES

On the basis of the tests just described the 90 cultures were gone over and 18 were chosen as representative of all the types found in the larger group. These were replated and new colonies picked. The purified cultures were inoculated into 10 c. c. of 1 per cent yeast-water solution of melezitose, raffinose, and salicin, and after seven days' incubation titrated for acid. Their ability to form mannitol from fructose was also determined by fermenting 75 c. c. of 2 per cent fructose-yeast water containing an excess of $CaCO_3$. After seven days the fermented medium was carefully decanted from the excess $CaCO_3$ and evaporated to 5 to 10 c. c. on the steam bath and then allowed to go to dryness at room temperature. If the culture is a good mannitol former, crystals of mannitol will appear throughout the residue and frequently extend up the sides of the beaker. The test is usually very definite, but if doubt existed the fermentation was repeated. The cultures in which no crystals appeared the second time were acidified with H_2SO_4 and extracted four times with ether to remove lactic acid, which tends to hide the mannitol crystals. The residue from the ether extraction was then extracted six times with hot 80 per cent alcohol and the extract concentrated to a small volume on the steam bath and then evaporated spontaneously at room temperature. If no mannitol crystals appeared the cultures were classed as nonmannitol-forming. The results of this test and the other characteristics of the 18 cultures are summarized in Table 7. This list includes cultures which ferment both pentoses, some of which ferment arabinose but not xylose, and some which ferment neither. Only 3 cultures were obtained which fermented xylose but not arabinose. Such a microorganism has been reported in a previous publication (*21*), but the arabinose-negative, xylose-positive type is much less common than the reverse combination.

Only four cultures were found to ferment melezitose, and all of these were mannitol-formers. The converse is not true, however; several cultures formed mannitol but did not ferment melezitose.

If 25 c. c. of 0.1 N acid be taken as indicating fermentation, more than half of the 18 cultures attack raffinose and salicin. According to Orla-Jensen (*13*), the ability to ferment salicin is rare among the lactic-acid bacteria of green vegetable matter.

TABLE 7.—*Fermentation characteristics of selected cultures*

Culture No.	Gas	Litmus milk	Mannitol formation	0.1 N acid in 100 c. c. of culture						
				Arabinose	Xylose	Glucose	Fructose	Melezitose	Raffinose	Salicin
				C. c.	*C. c.*	*C. c.*	*C. c.*	*C. c.*	*C. c.*	*C. c.*
29–7	−	−	−	28	21	82	99	16	16	56
36–6	−	+	+	23	34	108	88	75	89	74
54–10	−	−	−	74	24	63	62	24	33	25
25–7	−	+	−	76	70	67	72	17	19	33
32–2	+	+	+	70	70	73	58	17	58	55
31–2	−	+	−	10	28	55	12	8	18	29
64–2	−	+	+	69	30	100	91	67	85	80
57–8	+	−	+++	67	60	88	47	17	25	18
58–8	−	+	+	106	32	104	104	84	63	88
22–10	+	+	−	34	40	53	8	23	40	27
46–2	+	−	+++	51	115	96	58	19	18	20
51–9	+	+	+	86	50	80	53	15	43	80
22–8	+	−	++	72	46	63	93	17	48	57
55–9	+	+	++	80	80	88	54	20	42	48
48–3	+	−	+++	134	107	82	50	28	22	23
36–4	+	+	+++	108	133	100	51	19	20	21
64–9	+	+	++	114	106	92	87	19	62	23
26–8	+	−	++	64	59	57	90	17	38	52

A large majority of the organisms are mannitol formers but vary in the quantity which they produce. Those forming a small quantity are indicated with one plus sign in the table, while those forming a large quantity are indicated with two or three plus signs. Three of the mannitol formers, Nos. 36–6, 64–2, and 58–8, were conspicuous because of the large deposit of calcium salts which separated out with the mannitol crystals. They also failed to form gas from either glucose or fructose. The tests for gas and mannitol formation were repeated twice to make sure there was no error. The quantity of mannitol was small but unmistakable. Apparently the formation of mannitol is a property which varies from traces to 50 to 60 per cent of the fructose consumed. In 1921 Kayser (*11*) reported a similar case of mannitol formation by lactic-acid bacteria which did not form any gas.

Several of the cultures are noteworthy in that they produce no turbidity in the media. Floccules appear on the sides of the tube or settle to the bottom, leaving the medium perfectly clear. Cultures 25–7 and 64–9 were conspicuous in this respect. Considerable importance has been attached to the property of floccule formation in a recent classification of lactic-acid bacteria by Van Steenberge (*20*)

QUANTITATIVE DETERMINATION OF THE FERMENTATION PRODUCTS FROM ARABINOSE, FRUCTOSE, AND GLUCOSE

Six of the mannitol formers were selected for quantitative work. Differences in the fermentation of the sugars formed the basis of the selection. From the following summary it can be seen at a glance what the differences are. The minus sign signifies that there was little or no fermentation; the plus sign indicates good fermentation, and the double plus very good fermentation.

Culture No.	Arabinose	Xylose	Glucose	Fructose	Melezitose	Raffinose	Salicin
36–6	−	−	+	+	+	+	+
64–2	+	−	+	+	+	+	+
57–8	+	+	+	+	−	−	−
36–4	++	++	+	+	−	−	−
55–9	+	+	+	+	−	+	+
64–9	+	+	+	+	−	+	−

The cultures were inoculated into 300 c. c. of sugar-yeast-water media containing about 2 per cent of the sugar. The fermentation flask was equipped with a trap and absorption bottle for collecting the CO_2. Sterile bromo-cresol purple was added to the flask, and as fermentation proceeded the acid which formed was neutralized with sterilized 1 N NaOH. When acid production ceased the culture was acidified with H_2SO_4 and aspirated for 30 minutes to remove and absorb any traces of CO_2 in the flask. The KOH solution was made to a definite volume and analyzed for CO_2 by the Van Slyke (*22*) apparatus. The fermented medium was made to 400 c. c. and aliquots analyzed for unfermented sugar, alcohol, volatile acid, nonvolatile acid, and mannitol by methods already described (*18*, *19*, *5*, *15*). The data are given in Table 8.

TABLE 8.—*Fermentation products of arabinose, glucose, and fructose*

[Calculated for 100 c. c. of culture]

Culture No.	Age	Sugar		Acids		Alcohol as ethyl	Carbon dioxide	Mannitol	Total products
		Kind	Amount fermented	Volatile as acetic	Nonvolatile as lactic				
	Days		*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
57–8	7	Arabinose	1.823	0.841	0.937	0.012	0.040		1.830
36–4	7	do	1.829	.806	.983	.005	.036		1.830
55–9	12	do	1.138	.427	.674	.006	.026		1.133
58–8	11	do	1.938	.347	1.412	.008	.047		1.814
64–9	8	do	1.864	.379	1.485	.005	.041		1.910
64–2	8	do	1.938	.505	1.319	.008	.062		1.894
57–8	36	Glucose	1.225	.085	.438	.248	.270		1.041
64–9	34	do	1.700	.133	.620	.297	.365		1.415
36–4	15	do	.747	.052	.281	.141	.116		.590
55–9	15	do	1.497	.047	.658	.319	.314		1.338
64–2	34	do	1.110	.051	.846	.008	.042		.947
36–6	36	do	1.940	.066	1.728	.019	.046		1.859
57–8	12	Fructose	1.550	.288	.377	.055	.223	0.516	1.459
64–9	14	do	1.615	.257	.677	.129	.344	.232	1.639
36–4	12	do	1.580	.299	.580	.082	.246	.173	1.380
55–9	8	do	1.068	.234	.438	.051	.159	.103	.985
36–6	17	do	1.550	.044	1.429	.029	.066	?	1.568
64–2	14	do	1.615	.052	1.558	.013	.048	?	1.671

FERMENTATION OF ARABINOSE

This sugar was fermented very vigorously by all the cultures with the exception of No. 36–6 which does not attack this pentose. The principal products formed were volatile and nonvolatile acids. Only traces of CO_2 and alcohol were formed.

There was great variation in the quantity of products formed. The volatile acid comprised from 19 to 47 per cent and the nonvolatile acid from 50 to 78 per cent. On the basis of arabinose fermented, the lactic acid amounts to more than 70 per cent of the sugar. If more than 60 per cent of the sugar is converted into lactic acid, it is necessary to assume that parts of two molecules of arabinose are drawn upon to produce the extra 10 or more per cent of lactic acid. Only one 3-carbon compound can be obtained per molecule of pentose unless the remaining 2-carbon portions are first combined and then broken down to yield a 3-carbon compound such as lactic acid. A synthesis as well as a cleavage must therefore be brought about by the

bacteria. Cultures 58–8, 64–2, and 64–9 are all outstanding because of their ability to convert more than 60 per cent of the arabinose into lactic acid.

Fermentation of Glucose

The six cultures fall into two groups. Nos. 36–6 and 64–2 are straight lactics, while the other four form considerable quantities of alcohol and carbon dioxide with a small amount of volatile acid. These four cultures break up the glucose approximately as follows: $C_6H_{12}O_6 = C_3H_6O_3 + C_2H_5OH + CO_2$.

The actual figures show a slight departure from the theoretical, but in most cases this is less than 5 per cent.

Fermentation of Fructose

Fructose was rapidly and almost completely destroyed. Large quantities of lactic acid were formed by Nos. 36–6 and 64–2, but no mannitol could be found. Whether mannitol is formed under the somewhat acid conditions of the fermentation or whether its presence could not be detected because of interfering substances is not known. Mannitol was formed in the presence of $CaCO_3$, but the amount was too small to determine quantitatively. The remaining four cultures were very similar in the products formed. The volatile acids varied from 16 to 24 per cent.

Mannitol, carbon dioxide, and acetic acid production run parallel to one another and inversely to the production of lactic acid. The cultures which produced much mannitol also produced large quantities of CO_2. Appreciable quantities of ethyl alcohol were formed only by such cultures as produced considerable quantities of mannitol.

Form of Lactic Acid Produced

The form of lactic acid is readily identified by the water of crystallization of its zinc salt. Table 9 gives the data for these salts. Most of the lactic acid produced by these organisms was inactive. Culture 55–9 differs from the other cultures in producing an excess of one form of lactic acid. The water of crystallization is intermediate between that for inactive and active zinc lactate. To determine which enantiomorph was in excess, the zinc salts were examined in a polariscope. The first crop from arabinose and fructose was tested in each case and both were found dextrorotatory. Since the rotation of the zinc salt is opposite to that of the free acid, evidently culture 55–9 produces more *l*- than *d*-lactic acid.

TABLE 9.—*Forms of lactic acid produced by various cultures*

Culture No.	Sugar	Zinc lactate			Extracted acid recovered as zinc salt
		Crop No.	Weight	Water of crystallization [a]	
			Gms.	*Per cent*	*Per cent*
64-2	Glucose	1	0.8173	18.1	65
64-9	do	1	.5711	18.1	60
36-6	do	1	2.1144	18.1	81
57-8	do	1	.4116	18.0	59
64-2	Fructose	1	2.0902	18.1	81
64-9	do	1	.6636	17.7	54
		2	.1284	17.5	
36-4	do	1	.5875	18.2	52
		2	.0666	18.1	
36-6	do	1	2.2923	18.2	78
55-9	do	1	.1022	15.4	40
		2	.1913	13.8	
57-8	do	1	.4626	17.8	51
		2	.0436	17.9	
64-2	Arabinose	1	1.5252	18.4	61
		2	.1281	17.8	
64-9	do	1	1.7636	18.2	96
36-4	do	1	1.1992	18.5	88
		2	.1631	17.2	
55-9	do	1	.7467	14.3	68
57-8	do	1	1.0091	18.5	92
		2	.3527	18.5	

[a] Theory for H_2O in $Zn(C_3H_5O_3)_2+3H_2O=18.2$; theory for H_2O in $Zn(C_3H_2O_3)_2+2H_5O=12.9$.

SUMMARY

The acidity, temperature, and number of bacteria in four vats of commercial sauerkraut were determined at different intervals for a period of more than five months. There was a slow but regular increase in acid which, calculated as lactic, was equivalent to about 1 per cent after three months, and about 1.3 per cent after five months. The temperature in all of these vats was unusually low. At no time did it rise above 10.5° C., and this fact was undoubtedly responsible for the slow development of acid. There was a rapid increase in the number of bacteria from the second to the twentieth day, after which there was a big drop in the number of microorganisms.

Pure cultures were picked from each plating until a total of 370 were obtained. Most of these organisms fermented arabinose, xylose, and fructose but produced little change in litmus milk. Practically all were catalase-negative.

The fermentation characteristics of representative cultures of the 370 were studied further. A few of these were coccus forms but most of them were rods. The large majority were Gram-positive and catalase-negative. About two-thirds formed gas from glucose and approximately the same number formed mannitol from fructose. Several new strains of mannitol-forming bacteria were found.

Six of the mannitol-forming cultures were used for the quantitative determination of their fermentation products. Lactic acid was the principal product, and varied from 36 per cent in the case of glucose to 79 per cent in the case of arabinose. Such a large percentage of lactic acid can not be obtained by direct cleavage of the pentose molecule, but must involve synthesis of portions from two or more molecules. In most cases approximately equal quantities of *d*- and

l-lactic acid were produced. One culture, No. 55–9, produced an excess of the *l* form.

Besides lactic acid, acetic acid, and ethyl alcohol, carbon dioxide, and mannitol were produced in quantities which varied with the sugar and the culture used.

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